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Elevated Glycosyltransferase Activities in Infected or Traumatized Hosts: Nonspecific Response to Inflammation

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Streptococcus pneumoniae infection leads to multifold increases in sialyltransferase, galactosyltransferase, α_2-fucosyltransferase, and α_3-fucosyltransferase activity of rat liver. Such changes may reflect an increased demand for glycosylation of acute-phase proteins synthesized and secreted by the liver during inflammatory processes. Serum sialyltransferase became elevated in bacteria-infected or burned rats and sandfly fever-infected humans, but did not correlate with acute-phase serum protein changes. These data suggest that nonparenchymal liver cells, such as macrophages, may contribute substantially to elevated sialyltransferase activity in the circulation during infection and, as such, represent a general host response to infection and tissue trauma.

The glycosyltransferases that add sugars to nascent glycoconjugates have gained increasing interest during recent years because of their apparent association with the neoplastic state (9, 26, 32). Increases in the activity of sialyltransferase, galactosyltransferase, α_2-fucosyltransferase, and α_3-fucosyltransferase have been found in sera of cancer patients (14, 21, 27). The origin of the glycosyltransferases in the blood of these patients is unclear. Neoplastic cells have been proposed as a source of the enzymes, but secretion by nontumor cells, such as the liver, has not been excluded (5, 15, 19, 25).

Because there is increased glycoprotein synthesis by the liver during certain neoplastic and inflammatory disease states (16, 28, 30), we considered the possibility that elevated serum levels of glycosyltransferases may be characteristic of a variety of inflammatory disease processes rather than specific to neoplasia. Accordingly, we undertook a study to determine glycosyltransferase activity in infected or traumatized hosts.

MATERIALS AND METHODS

Experimental models. Male Sprague-Dawley rats from Charles River Laboratories, weighing 200 to 250 g, were inoculated subcutaneously at appropriate intervals with 10^8 virulent (infected) or heat-killed (control) *Streptococcus pneumoniae* type I organisms (U.S. Army Medical Research Institute of Infectious Diseases strain). Groups of rats were killed at 24 and 48 h after inoculation; these rats had been fasted for 48 h at time of their death. Liver homogenates (10%, wt/vol, in 0.9% NaCl) and serum were prepared and assayed for glycosyltransferases. Fever, bacteremia, α_2-macrofetoprotein, and serum Zn and Cu were monitored to assess development of the septic state.

Male albino rats purchased from Holtzman Co. (Madison, Wis.) were used for the burn model. A 30% total body surface, full-thickness burn of the dorsum was achieved by immersing anesthetized, shaved rats, placed in a mold to define the extent of injury, in boiling water for 10 s. At selected intervals up to 29 days postburn, control and burned rats in groups of four were bled, and serum was prepared and assayed for glycosyltransferases and α_2-macrofetoprotein determinations.

Male human subjects were recruited from personnel participating as the Medical Research Volunteer Subject group at the U.S. Army Medical Research Institute of Infectious Diseases. They were informed fully of the purpose, risks, and specific experimental details before volunteering. The primary purposes of the sandfly fever study were to evaluate the interactions between physical activity and a mild self-limited virus infection; serum for the present investigation was collected as a secondary objective. Seven men were inoculated with 0.5 ml of diluted (1:10 in sterile physiological saline) human plasma previously shown to contain sandfly fever virus (31). Base-line physical examinations and laboratory tests were performed on an outpatient basis before virus inoculation and at selected intervals thereafter. All subjects were hospitalized for a 6-day period beginning day 2 after inoculation, then were followed on an outpatient basis for an additional period of 20 days. Venous blood samples were obtained at approximately 0730 h on the days indicated. Serum was prepared from a portion of each sample and analyzed for glycosyltransferases, haptoglobin, α_1-acid glycoprotein, and α_1-antitrypsin. Another portion was used to determine total and differential leukocyte counts.

Analytic methods. Galactosyltransferase and *N*-acetylglucosaminyltransferase were measured with ovalbumin as acceptor. Desialofetuin was the acceptor for sialyltransferase and α_3-fucosyltransferase, and desialodegalactofetuin served as acceptor for α_3-fucosyltransferase. Assay conditions were adapted from published isotopic procedures (2, 3, 29). Reaction cocktails (40 μl) were mixed with 20 μl of liver homogenate or

serum and incubated at 37°C. A blank was run simultaneously in which enzyme was replaced by water. After an appropriate period of incubation, reactions were terminated by pipetting 40 μl of the mixture onto strips of trichloroacetic acid-treated Whatman 3MM chromatography paper, washed, and counted by scintillation spectroscopy (17).

Serum Zn and Cu were determined by atomic adsorption spectrophotometry (20, 23). Acute-phase serum proteins were quantitated by an automated system for immunoassay by nephelometry, as described by Bostian et al. (6). Protein was assayed by the method of Lowry et al. (18).

RESULTS

Rats inoculated with 10^5 *S. pneumoniae* uniformly succumb to the infection with a mean time to death of about 66 h (17). Rats became febrile within 12 to 16 h and at 24 h showed characteristic signs of acute infection, including fever, bacteremia, modulation of the concentration of serum metals, and an increase in the acute-phase protein, α_2-macrofetoprotein (Table 1). Marked increases in activity of four liver glycosyltransferases were found 24 h after inoculation of bacteria (Table 2). At 48 h, the increase in total activity in liver varied from 230% for α_3-fucosyltransferase to 450% of control for sialyltransferase. The change in *N*-acetylglucosaminyltransferase activity was less pronounced than that of other glycosyltransferases. In serum, the activity of sialyltransferase and α_2-fucosyltransferase increased 13 and 2.3 times, respectively, but, in contrast to liver, *N*-acetylglucosaminyltransferase, galactosyltransferase, and α_3-fucosyltransferase activities were not changed.

Sandfly fever in men was characterized by a febrile response on days 3 and 4 after virus inoculation and a significant rise in the plasma levels of haptoglobin and α_1-acid glycoprotein on days 4 to 7 (Fig. 1). A significant increase in sialyltransferase activity was found only on days 4 and 5; in contrast, serum levels of α_2-fucosyltransferase were not altered.

In rats with 30% burns, α_2-macrofetoprotein was elevated through day 4 postburn. In contrast, serum sialyltransferase activity, but not α_2-fucosyltransferase, was increased throughout the entire acute and convalescent periods (Fig. 2).

DISCUSSION

The elevated glycosyltransferase values in livers of septic rats were not unexpected, considering the markedly increased rates of hepatic synthesis and secretion of serum glycoproteins which characterize an infectious process (6, 17, 24, 33). Clinical states associated with tissue trauma, including neoplasia, myocardial infarction, burns, and sterile inflammatory lesions, also engender a nonspecific anabolic response on the part of the liver, resulting in the synthesis of a class of glycoproteins collectively termed acute-phase globulins (17, 24, 28, 33). These proteins are thought to function in a compromised host to restrict tissue damage by inhibiting proteases released at inflammatory sites, to aid in wound healing, to modulate clotting, to activate phagocytes, to promote phagocytosis of microorganisms and necrotic tissue, and to modulate the immune response (8, 22). We propose that the increased activity of liver glycosyltransferases observed in this study represents a nonspecific host response to inflammation engendered by the greater demand for glycosylation of newly synthesized acute-phase globulins within the Golgi complex of liver cells.

The source of the increased serum glycosyltransferase activities in septic rats remains to be defined. It might be expected that some enzymes from within the Golgi complex of liver cells may be adventitiously secreted into the blood along

TABLE 1. *Parameters measured in rats inoculated with S. pneumoniae*

Time	Rats	Parameter								
		Body wt loss (g)	Rectal temp (°C)	Bacteremia (CFU[a] × 10^6 per ml of blood)	Serum macrofetoprotein (units)	Serum metals (μg/dl)		Serum protein (mg/ml)	Liver	
						Zn^{2+}	Cu^{2+}		Protein (mg/g of liver)	Wt (g)
24 h	Control	42.8 ± 0.9	36.9 ± 0.2	0	4.7 ± 0.4	128 ± 4	138 ± 5	84.5 ± 1.1	285 ± 10	6.07 ± 0.17
	Infected	50.7 ± 1.1[b]	39.1 ± 0.1[b]	1.23 ± 0.62[b]	21.5 ± 6.7[c]	44 ± 4[b]	255 ± 11[d]	75.0 ± 2.3[c]	253 ± 7[c]	8.28 ± 0.11[b]
48 h	Control	47.3 ± 0.8	36.6 ± 0.1	0	5.0 ± 0	160 ± 5	140 ± 3	83.0 ± 2.1	285 ± 6	6.39 ± 0.09
	Infected	44.0 ± 2.5	38.7 ± 0.3[b]	1.30 ± 0.46[b]	154.0 ± 21.2[b]	43 ± 5[b]	181 ± 11[d]	63.5 ± 1.9[b]	238 ± 7[b]	9.05 ± 0.13[b]

[a] Bacteremia is reported as colony-forming units (CFU) per milliliter of whole blood ± standard error. Mean values, six rats per group, were compared by Student's *t* test.

[b] $P < 0.001$.

[c] $P < 0.005$.

[d] $P < 0.001$.

[e] $P < 0.05$.

TABLE 2. *Serum and liver glycosyltransferase activities in rats inoculated with S. pneumoniae*[a]

Time	Rats	Sialyltransferase (pmol/min per ml)		Galactosyltransferase (nmol/min per ml)		*N*-Acetylglucosaminyltransferase (pmol/min per ml)		α_3-Fucosyltransferase (pmol/min per ml)		α_2-Fucosyltransferase (pmol/min per ml)	
		Serum	Liver	Serum	Liver	Serum	Liver	Serum	Liver	Serum	Liver
24 h	Control	2.90 ± 0.17	829 ± 92	0.86 ± 0.05	162 ± 4	6.13 ± 0.26	9,400 ± 350	0.108 ± 0.009	27.8 ± 10.2	0.062 ± 0.008	2.29 ± 0.15
	Infected	7.62 ± 0.27[b]	3,102 ± 139	1.00 ± 0.04	516 ± 17[b]	5.98 ± 0.32	8,760 ± 360	0.177 ± 0.038[c]	84.2 ± 15.2[d]	0.077 ± 0.012	3.48 ± 0.13[b]
48 h	Control	1.51 ± 0.48	862 ± 75	1.05 ± 0.4	147 ± 11	6.37 ± 0.29	6,990 ± 150	0.097 ± 0.012	34.4 ± 11.5	0.087 ± 0.007	1.76 ± 0.11
	Infected	19.6 ± 3.4[b]	3,880 ± 211[b]	0.96 ± 0.07	693 ± 39[b]	6.72 ± 0.29	9,250 ± 260[b]	0.238 ± 0.020[b]	118.1 ± 13.6[b]	0.083 ± 0.009	4.09 ± 0.20[b]

[a] Transferase activities are reported as picomoles or nanomoles per minute per milliliter of serum or total liver. Specific activities may be calculated from data given in Table 1. Values are means ± standard error of six rats compared by Student's *t* test.

[b] $P < 0.001$.

[c] $P < 0.05$.

[d] $P < 0.025$.

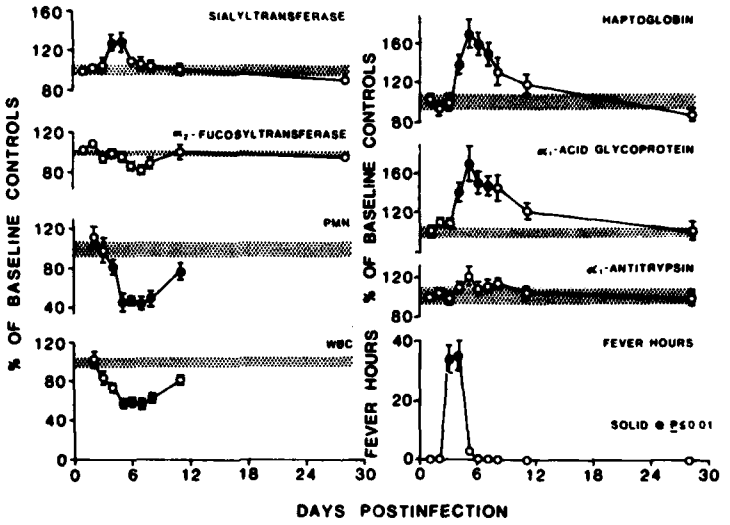

FIG. 1. *Measured parameters during sandfly fever infection in men. Values are mean ± standard error of seven individuals, expressed as a percentage of preinfection values. Shaded areas are the means ± standard error of the base-line values before exposure. Solid circles indicate values significantly different ($P < 0.01$) from the mean base-line data obtained before infection. Fever hours are the product of degrees Farhenheit above body temperature of 99°F (ca. 37.2°C) multiplied by duration in hours. PMN, polymorphonuclear leukocytes. WBC, leukocyte count.*

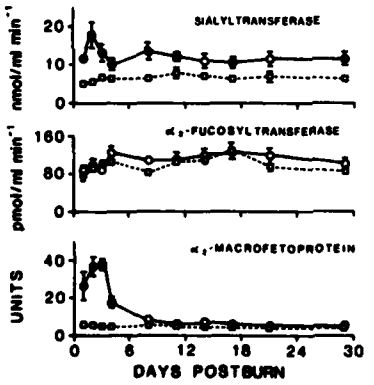

FIG. 2. *Glycosyltransferase activities and α_2-macrofetoprotein level in serum of control (dashed line) and burned rats (solid line). Each point represents the mean ± standard error of four rats. Solid symbols indicate values significantly different ($P < 0.005$) from control values. α_2-Macrofetoprotein units are expressed as a percentage of a standard reference serum obtained from turpentine-inflamed rats.*

with newly completed acute-phase glycoproteins. However, since sialyltransferase, galactosyltransferase, and *N*-acetylglucosaminyltransferase in rat liver Golgi share very similar compartments (R. Bretz, H. Bretz, and G. E. Palade, J. Cell Biol. 79:247a, 1978), a selective release of just sialyltransferase would appear unlikely. Furthermore, the temporal profile of sialyltransferase activity in serum of virus-infected humans and burned rats differed noticeably with respect to changes in serum levels of measured acute-phase proteins. These data argue against a selective simultaneous secretion of glycosyltransferases and acute-phase glycoproteins by the liver and support the observation that elevations of sialyltransferase activity in liver and in the circulation are independently regulated (12).

The purposeful secretion of certain glycosyltransferases into the circulation by other cells cannot be excluded. Phagocytic cells are one possible source of the enzymes. Mouse peritoneal macrophages have been found to secrete appreciable quantities of glycosyltransferases (7). In addition, stimulated rabbit alveolar macrophages have elevated levels of sialyltransferase and α_2-fucosyltransferase (11). It is conceivable that activation of macrophages and other phagocytic cells during infection or a necrotic process may result in the release of selected glycosyltransferases into the circulation.

The observation that elevated serum activities of sialyltransferase, α_2-fucosyltransferase, and other glycosyltransferases are associated with neoplastic diseases has led to proposals that the blood values of certain glycosyltransferases may be used in the diagnosis of malignancy, as well as an indicator of successful tumor therapy (1, 2, 4, 10, 13). This conclusion is mitigated by present

observations, suggesting that changes in serum glycosyltransferase activities may also occur as nonspecific consequences of the generalized host response to cellular damage and inflammation. Certain patterns of serum glycosyltransferase levels may yet be recognized to have diagnostic significance. However, further characterization of enzyme activities during specific pathophysiological events is required before the full diagnostic potential of serum glycosyltransferase activities can be realized.

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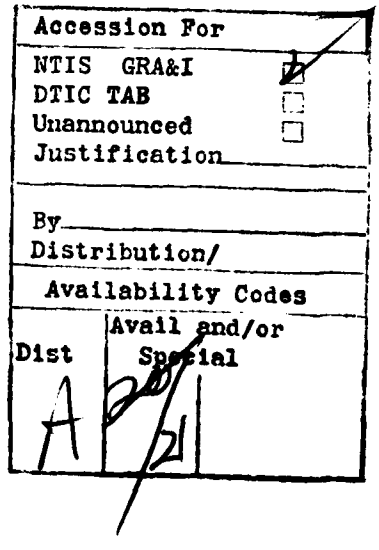
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